REMARKS

Claim 7, 8, 10, 12, 14-18, 20 and 22-31 are currently pending. It is asserted that 8, 12, 14, 22 and 23 have been withdrawn. Thus, these claims are still pending.

It is still Applicant's position that claims 8, 12 and 22 constitute linking claims (linked to claim 7). Further, it is Applicant's position that claim 23 should also be considered to be a linking claim (linked to claim 7). Again, Applicant respectfully requests that the Restriction Requirement be reconsidered.

As will be discussed in further detail below, claims 7, 24, 25-28 have been amended to more distinctly claim that which Applicant regards as his invention and to advance prosecution. Claim 29 has also been canceled to advance prosecution. These amendments and the cancellation of claim 29 are not acquiescence to the Examiner's position and Applicants reserve the right to file subsequent continuation and/or divisional applications on subject matter originally recited in the claims.

1. Claim Objections

Claim 25 is objected to since it depends from claim 24 that is drawn to "An isolated nucleic acid molecule". It is suggested that claim 25 should recited "The isolated nucleic acid molecule of claim 24" not "sequence of claim 24". In response, claim 25 has been amended accordingly.

Claim 29 is objected to because "SSC" is mistyped. As noted above, claim 29 has been canceled.

In view of the amendment of claim25 and cancellation of claim 29, the objections have been overcome.

2. The Rejections Under 35 U.S.C. §112, First Paragraph-Written Description

2.1 Claims 24, 25, 28 and 29

Claims 24, 25, 28 and 29 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the

application was filed, had possession of the claimed invention. Specifically, the Office Action states

> Claim 24 is drawn to a "nucleic acid molecule consisting of at least 20 nucleotides unique to a reverse or forward strand of a contiguous exon-intron region or a contiguous intron-exon region". Thus, it is drawn to any nucleotide sequence, because it is not specified to which sequence an exon-intron and intron-exon regions belong. The specification has support for said regions in SEQ ID N0:4 but not in other sequences.
>
> Claims 25, 28 and 29 recite 20-5000 nucleotides. The specification provides support for the discrete numbers of nucleotides such as 20, 30, 50, 100, ... 5000 (page 3, lines 19-22; page 9, line 34 through page 10, line 2). However, the examiner is unable to locate adequate support in the specification for a fragment of any length that is more than 20 and less than 5000 nucleotides. Thus, there is no indication that exon- intron or intron-exon regions of any genomic sequence as well as fragments of 20-5000 nucleotides of SQ ID NO: 4 were within the scope of the invention as conceived by Applicants at the time the application was filed.

In response, Applicant has amended claim 24 to recite that it is directed to an exon-intron and intron-exon region of SEQ ID NO:4.

Applicant traverses the rejection with respect to claims 25 and 28. The recitation of these discrete numbers in the instant application provides sufficient support. This issue was discussed in MPEP §2163.06 (pg 2100-182), copy attached hereto as Appendix A when referring to *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976). In *Wertheim,* a limitation in a claim "between 35% and 60%" was found to meet the written description requirement since the specification included a range of "25%-60%" and specific examples of "36%" and "50%". An analogous situation exists in this instance. Clearly, there is a recitation of fragment lengths between 20 and 5000 in the specification. Therefore, the written description requirement is met. Applicant notes that claim 29 has been canceled.

In view of the amendment of claim 24, the cancellation of claim 29 and the above arguments, Applicant asserts that the rejection under 35 U.S.C. §112, first paragraph (written

description) has been overcome. Therefore, Applicant respectfully requests that the rejection be withdrawn.

2.2 Claims 7, 10, 15-18, 20, 24-28, 30 and 31

It is asserted with respect to claims 7, 10, 15-18, 20, 24-28, 30 and 31 that "unique" does not mean identical. Therefore, in the Examiner's view, claims 7, 10, 15-18, 20 and 24-31 encompass structurally and/or functionally diverse nucleotide molecules.

Applicant respectfully traverses the rejection. In response, Applicant asserts that the definition of "unique" is well known in the art. For example, it is stated in http://big.mcw.edu/display.php/2001.htm that a unique sequence is a sequence that occurs once only in a haploid genome. However, in order to advance prosecution, Applicant has amended claims 7, 24, 26 and 27 to recite that the isolated nucleic acid molecule consists of a reverse or forward strand of a region of SEQ ID NO:4 to more distinctly recite the subject matter encompassed by these claims. As noted above, claim 29 has been canceled.

Further assertions were made with respect to claims 24 and 25. Specifically, it is stated

> Claim 24 is drawn to a "nucleic acid molecule consisting of at least 20 nucleotides unique to a reverse or forward strand of a contiguous exon-intron region or a contiguous intron-exon region.
>
> Claim 25 depends from claim 24 and is drawn to "nucleic acid molecule [that] is 20-5000 nucleotides in length and contains two nucleotides at the specified positions of SEQ ID N0:4. Thus, claim 24 encompasses any nucleotide sequence and claim 25 is limited to any sequence of 20-5000 nucleotides that hybridizes under unspecified moderate stringency conditions to SEQ ID N0:4. The recitation of nucleotides at the two specific positions of SEQ ID N0:4 practically does not limit the structure of said 20-5000 nucleotides because any two nucleotides can be found in any sequence of 20-5000 nucleotides. It is noted that claim 25 is not drawn to a fragment of SEQ ID N0:4 consisting of 20-5000 nucleotides.
>
> Claim 29 is drawn to "An isolated nucleic acid molecule containing between 20 and 5000 nucleotides or its reverse strand that hybridizes at 55C,5xSSC to a non- coding region of SEQ ID N0:4. It is noted that "containing" is open language and

the length of the entire molecule as well as the composition of the flanking sequences are not limited.

In response, Applicant notes that claim 24 has been amended to be directed to an isolated nucleic acid molecule 20-5000 nucleotides in length **consisting of** a reverse or forward strand of a contiguous exon-intron region or a contiguous intron-exon region **of SEQ ID NO:4.** Given that claim 25 depends from claim 24, amendments to claim 24 would also overcome the rejection of claim 25. Furthermore, claim 29 has been canceled.

The other claims mentioned in the rejection, claims 10, 16-18, 20, 27-28 and 30-31 depend from claim 7. Therefore, arguments made with respect to claim 7 apply. Claim 25 depends from claim 24. Therefore arguments made with respect to claim 24 applies.

In view of the above arguments and amendments of claims 7, 24 and 25, Applicant asserts that the rejections of claims 7, 10, 15-18, 20, 24-28, 30 and 31 have been overcome. Therefore, Applicant respectfully requests that the rejections be withdrawn.

3. The Rejections Under 35 U.S.C. 112, First Paragraph, Lack of Enablement

Claims 7, 10, 15-18, 20 and 24-31 have been rejected under 35 U.S.C. 112, first paragraph as lacking enablement. It is asserted that the specification is enabling for a non-coding region of at least 20 nucleotides or 20-5000 nucleotides of SEQ ID NO:4 but not for nucleotides that are unique or hybridize at 55C, 5X SSC to a non-coding region, including the splice junction and have no known function.

In response and in order to advance prosecution, Applicant notes that claims 7, 24, 26 and 27 have been amended to be directed to isolated nucleic acid molecules **consisting of** regions of SEQ ID NO:4. Therefore, it would satisfy the Examiner's criteria with respect to enabling disclosure. Claims 10, 15-18, 20, 24-28, 30 and 31 depend from claim 7 and claim 25 depends from claim 24. Claim 29 has also been canceled to advance prosecution.

In view of the amendments of claims 7, 24, 26 and 27, the cancellation of claim 29 and the above arguments, Applicant asserts that the rejections under 35 U.S.C. §112, first paragraph have been overcome. Therefore, Applicant respectfully requests that the rejections be withdrawn.

4. The Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 7, 10, 15-18, 20 and 24-31 have been rejected under 35 U.S.C. 112, second paragraph as being indefinite. The Examiner particularly took issue with the use of the terms "unique", "non-coding region" and "a contiguous exon-intron region or a contiguous intron-exon region".

Applicant respectfully traverses the rejection. However, in order to advance prosecution, claims 7, 24 and 26-27 have been amended. Specifically, the terms "unique", "non-coding region" and "a contiguous exon-intron region or a contiguous intron-exon region" have been removed from claim 7. The terms "exon-intron" region and "intron-exon region" remain, but the term "non-coding" has been removed in claim 24. However, given that claim 24 is directed to 20-5000 nucleotide fragments, it would not encompass the entire chromosome 12. The meanings of these terms are self-evident. Applicant further notes that in claim 24 the templates are SEQ ID NO:4 and its reverse strand. As noted above, claim 29 has been canceled.

In view of these claim amendments, Applicant asserts that the rejections under 35 U.S.C. 112, second paragraph have been overcome. Therefore, Applicant respectfully requests that the rejection be withdrawn.

5. The Rejections Under 35 U.S.C. 102(b)

Claims 7, 10 and 15 are rejected under 35 U.S.C. 102 (b) as being anticipated by Muzny et al. The Office Action states:

> Muzny et al. (GenBank accession AC025423, March 9, 2000) teach the sequence of human chromosome 12 comprising the sequence of SEQ ID NO: 4. Said sequence is of at least 20 nucleotides and is unique to a contiguous exon-intron or intron-exon region of SEQ ID NO:4. It would hybridize to its one fragment that is a non-coding region, including an exon-intron or intron-exon region.
>
> Claim 15 is included herein because "A kit" can be construed as a preamble that does not limit the scope and has no patentable weight.

It is further stated in the Office Action that claim 29 would not be anticipated since it contains an upper limit.

Applicant traverses the rejection. A human genomic sequence does not in and of itself imply the presence of a gene, nor does it identify a gene if present in the sequence. However, in order to more distinctly claim the subject matter of the invention and to advance prosecution, Applicant has amended claim 7 to recite that the nucleic acid molecule is between 20 and 51,039 nucleotides. This is shorter than the AC025423 which is >150 kb. Furthermore, claim 7 only encompasses forward and reverse strands of regions of SEQ ID NO:4. Applicant notes that claims 10 and 15 depend from claim 7 and would also not be anticipated by Muzny et al.

In view of the above arguments, the amendment of claim 7 and new claim 24, Applicant asserts that the rejection under 35 U.S.C. §102(b) has been overcome. Therefore, Applicant respectfully requests that the rejection be withdrawn.

6. The Rejections Under 35 U.S.C. §103(a)

Claims 7, 10, 15-20 and 24-31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Muzny et al, in view of Vogelstein et al.

> The teachings of Muzny et al are outlined above.
>
> Vogelstein et al (US Patent 5,411,860 GenBank accession NM_002392) teach cloning functional expression and chromosomal localization of human mouse double minute (MDM2) homolog. They teach cDNA (SEQ ID NO: 1) encoding human MDM2 homolog (SEQ ID NO: 2) that is 100% identical to the human MDM2 homolog of the instant invention (SEQ ID NO: 2). Using a labeled probe, they localized the gene encoding said human MDM2 homolog to chromosome 12q12-14 (citations omitted).
>
> It would have been obvious to one of ordinary skill in the art at the time the invention was made to use said cDNA to identify the genomic DNA that encodes the human MDM2 homolog of SEQ ID NO: 2 on chromosome 12q12-14. The motivation is provided by Vogelstein et al. who teach that it binds to oncogene p53 and is diagnostic of tumorogenesis. The state of the art provides various techniques for obtaining genomic DNA using cDNA probes that are usually labeled. The comparison of genomic and cDNA would result in the identification of non-coding regions. One of ordinary skill in the art would have been motivated to use said non-coding regions or fragments thereof of at least 20 nucleotides for detecting variants of

chromosome 12q12-14 from genomic nucleotide samples from an individual, for example. As a matter of convenience a non-coding region such as a splice junction or fragments thereof can be present in a kit or on a solid support. Further, said support can be a microarray to a customary use of nucleic acid molecules in the art.

It is further stated in the Office Action that

> With regard to the 103(a) rejection, Applicant argues that "Vogelstein et al. merely discloses the cDNA sequence of MDM2; it contains 2372 nucleotides. Muzny et al. discloses the sequences of AC025423 (150,579 nucleotides). Therefore, the cDNA constitutes only 1.6% of the sequence present on AC025423. No direction is provided in these references regarding the genomic organization of the MDM2 gene and particularly, the number and size of exon and intron sequences, location of exon-intron and inton-exon regions and the size of the 5' and 3' noncoding regions. There was also no indication provided in the cited references regarding the position of the noncoding sequences and particularly, contiguous exon-intron or intron-exon regions within AC025423 with respect to the MDM2 gene. Muzny et al. did not recognize that the gene was present in this clone. The human MDM2 genomic sequence was unexpectedly found to contain at least 10 exons. There is a vast range in the size of the introns ranging from 126 bases to about 7 kB. There is also a significant range in the size of the exons, ranging from 51 bases to 573 bases. Again as noted above, no teaching was provided with respect to the size or location of the noncoding or coding sequences of MDM2 within AC025423 (page 13). This is not persuasive because Muzny et al disclose the sequence of a human chromosome which should contain genes. Vogelstein et al localized the requisite gene to said chromosome. Further, while "the number and size of exon and intron sequences, location of exon-intron and inton-exon regions and the size of the 5' and 3' noncoding regions" were not disclosed, these references made them obvious. If they were disclosed, it would constitute anticipation.

> Applicant further argues that "There are a number of exon and intron sequences that are very small in size (see, for example, introns 25507-25384 (intron 5), 25287-21169 (intron 6) and exons 2 (36384-36310) and 29565-29615 (exon 4)). It is Applicant's assertion that one of ordinary skill in the art would not have a reasonable expectation of success of actually identifying these particular sequences, particularly, what

constitutes intron and exon sequences" (page 14).While these exons and introns are relatively small, there is no reason to believe that one of ordinary skill in the art at the time the invention was made would not localize them using available techniques.

An [sic] applicant further argues that "Applicant also notes that there has been a great deal of interest in the scientific community in MDM2 given its potential use as a diagnostic and therapeutic agent. This interest is summarized in the cited patent Vogelstein et al. However, there was absolutely no disclosure or suggestion of the genomic organization of MDM2 genomic DNA until the instant application was filed. An independent disclosure of the genomic organization of the MDM2 gene was not available until July 21, 2004, more than one year after the filing date of the instant application (Liang et al., 2004, Gene 338:217-223). The Court of Customs and Patent Appeals (CCPA) and its present successor, the Court of Appeals for the Federal Circuit (CAFC), have held the following considerations to be objective evidence of nonobviousness; longfelt need, commercial success, failure of others, copying and unexpected results. In re Sernaker, 702 F.2d 989, 217 U.S.P.Q. 1 (Fed. Cir. 1983); In re Imperato, 179 U.S.P.Q. 710 (CCPA 1973)". This is not persuasive because Applicant does not indicate which of the evidence such as long felt need, commercial success, failure of others, copying or unexpected results, is applicable in this case. Liang et al., supra, used previously known techniques to obtain the same results as disclosed in the instant application, i.e.. to localize exons and introns within the genomic sequence. .....

Applicants further argue that "A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out. *In re Deuel* 51 F.3d 1552, 34 U.S.P.Q.2d 1210 (Fed. Cir. 1995). Here, only a general incentive at best was provided" (page 15). This is not persuasive because in *In re Deuel* case, no nucleic acid was known whereas here it is known. The instant invention discloses the specific fragments of a known genomic nucleic acid sequence. The sequences of said fragments were known, the specification teaches the end points of said fragments, i.e. exons and introns. These end points are obvious.

Applicant respectfully traverses the rejection. First, Applicant would like to address the disclosure of Muzny et al. As previously discussed, Muzny et al. only disclosed the sequence of the clone AC025423. There was no indication at the time of the filing date of the instant application that AC025423 actually contained SEQ ID NO:4, more specifically a sequence encoding human MDM2. Actually, *contra* to assertions made in the Office Action, Muzny et al. did not sequence all of chromosome 12; they only sequenced the chromosome 12 BAC clone AC025423, less than 0.12% of the chromosome (about 157 Kb vs. about 132,000 Kb). The clone's sub-site on chromosome 12 apparently was not then known. Muzny et al. did not even suggest, must less state whether the AC025423 did or did not contain any gene. The determination of a sequence of genomic DNA does not necessarily imply the presence of a gene. This is because it is well known in the art and was certainly well known when the instant invention was made and as of the filing date of the instant application that most genomic DNA contains "junk" DNA. Most human genomic clones do not contain genes. When obtaining genomic clone sequences, one of skill in the art would not have any way of knowing whether or not it actually contains a gene(s). Even noting that a clone has a high GC content is a poor guide to a sequence's likely gene content, especially given the presence of pseudogenes. Therefore, *contra* to assertions made in the Office Action, disclosure of this particular sequence would not indicate to the skilled artisan that this clone would necessarily contain a gene.

As previously argued, there would not be any motivation to combine Muzny et al with Vogelstein et al. Muzny et al knew that clone AC025423 (from RP11-611o2) was from chromosome 12 but there is no evidence in the NCBI report of a sub-assignment to the p- or q-arm. Chromosome 12 is about 130 million base pairs long and is believed to contain several hundred genes (by analysis after 2001 and after the Applicant discovered the human MDM2 homologue gene). Further, there is no evidence that Muzny et al. knew whether the clone did or did not contain one or more genes and particularly whether it contained the gene encoded by SEQ ID NO:4. Vogelstein et al. placed the human MDM2 homologue gene at 12q12-14. Actually, this finding is incorrect. After the publication of Vogelstein, the gene was found to be located at 12q12-14, whereas the gene is actually several millions of base pairs away at 12q15 (see GeneCard attached hereto as Appendix B). There was actually a previous disclosure stating that the MDM2 was located between 12q14.3-15 (see, for

example, Andersen et al., 1996, Mammalian Genome 7:780-783 and Bureau, 1995, Genomics 28:109-112, submitted herewith as an IDS). However, given the conflicting locations published, one of ordinary skill in the art would not have known which location was actually correct. Clearly combining the disclosures of Muzny et al. with Vogelstein et al. would not have produced the claimed sequences, especially given Vogelstein's mistaken assignment of MDM2 to 12q12-14.

Applicant would further like to address the assertions made on pages 13-15 of the Office Action. Although it can be argued that techniques for making the discovery existed, it is Applicant's view that one would not have had a reasonable expectation of success of obtaining the claimed sequence. Using available techniques goes to "the manner in which the invention was made", an issue which, in Applicant's view has no bearing on obviousness. This is especially true given the publication of varying locations of the MDM2 gene. Additionally, Applicant notes that the gene sequence was not actually published until Liang et al. (2004), one year after Applicant's filing date. The dearth of knowledge regarding the MDM2 gene was actually admitted to by Liang et al. on the first page of his article where it was stated "Although the human MDM2 cDNA sequence has been reported, the genomic organization of the human gene has not been documented". Thus, the disclosure of Liang et al. would be evidence of "long felt need" with respect to secondary criteria of obviousness.

Finally, Applicant would like to address the assertion made on page 15 of the instant Office Action. *In re Deuel* was merely being cited for the teaching that "A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out. " However, although as noted in the Office Action, no nucleic acid sequence was known in *Deuel*, analogies may be drawn. In *Deuel*, the amino acid sequence was known yet the nucleic acid sequence was not known. It was held that given the large number of possible sequences, one of ordinary skill in the art would not be able to predict the correct sequence. It was actually stated in *Deuel* that "No particular one of these DNAs can be obvious unless there is something in the prior art to lead to the particular DNA and indicate that it should be prepared". In the instant application, there is a chromosomal DNA sequence. However, there is nothing in Vogelstein which would lead one of ordinary skill in the art to Muzny et al. to indicate that the claimed sequences could actually be found in that clone. Furthermore, Applicant again wishes to emphasize that a sequence in and of itself does not

predict whether or not a gene is present and certainly says nothing of which specific gene is present, if present at all. Mere inspection of the sequence of a gene-containing clone cannot reveal a gene's intron and exon organization.

7. Conclusion

In view of the foregoing, Applicants assert that the claims are now in condition for allowance. Early action to that end is respectfully requested. The Examiner is invited to contact the undersigned at (914) 712-0093 if she has any questions.

Respectfully submitted,

Date: 8/25/05

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APPENDIX A

inductive-deductive approach to arriving at a claimed subgenus, it cannot be said that such a subgenus is necessarily described by a genus encompassing it and a species upon which it reads." (emphasis added)). Each case must be decided on its own facts in terms of what is reasonably communicated to those skilled in the art. *In re Wilder*, 736 F.2d 1516, 1520, 222 USPQ 369, 372 (Fed. Cir. 1984).

III. RANGE LIMITATIONS

With respect to changing numerical range limitations, the analysis must take into account which ranges one skilled in the art would consider inherently supported by the discussion in the original disclosure. In the decision in *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976), the ranges described in the original specification included a range of "25%-60%" and specific examples of "36%" and "50%." A corresponding new claim limitation to "at least 35%" did not meet the description requirement because the phrase "at least" had no upper limit and caused the claim to read literally on embodiments outside the "25% to 60%" range, however a limitation to "between 35% and 60%" did meet the description requirement.

See also *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1328, 56 USPQ2d 1481, 1487 (Fed. Cir. 2000) ("[T]he specification does not clearly disclose to the skilled artisan that the inventors... considered the... ratio to be part of their invention.... There is therefore no force to Purdue's argument that the written description requirement was satisfied because the disclosure revealed a broad invention from which the [later-filed] claims carved out a patentable portion"). Compare *Union Oil of Cal. v. Atlantic Richfield Co.*, 208 F.3d 989, 997, 54 USPQ2d 1227, 1232-33 (Fed. Cir. 2000) (Description in terms of ranges of chemical properties which work in combination with ranges of other chemical properties to produce an automotive gasoline that reduces emissions was found to provide an adequate written description even though the exact chemical components of each combination were not disclosed and the specification did not disclose any distinct embodiments corresponding to any claim at issue. "[T]he Patent Act and this court's case law require only sufficient description to show one of skill in the . . . art that the inventor possessed the claimed invention at the time of filing.").

2163.06 Relationship of Written Description Requirement to New Matter

Lack of written description is an issue that generally arises with respect to the subject matter of a claim. If an applicant amends or attempts to amend the abstract, specification or drawings of an application, an issue of new matter will arise if the content of the amendment is not described in the application as filed. Stated another way, information contained in any one of the specification, claims or drawings of the application as filed may be added to any other part of the application without introducing new matter.

There are two statutory provisions that prohibit the introduction of new matter: 35 U.S.C. 132 - No amendment shall introduce new matter into the disclosure of the invention; and, similarly providing for a reissue application, 35 U.S.C. 251 - No new matter shall be introduced into the application for reissue.

I. TREATMENT OF NEW MATTER

If new subject matter is added to the disclosure, whether it be in the abstract, the specification, or the drawings, the examiner should object to the introduction of new matter under 35 U.S.C. 132 or 251 as appropriate, and require applicant to cancel the new matter. If new matter is added to the claims, the examiner should reject the claims under 35 U.S.C. 112, first paragraph - written description requirement. *In re Rasmussen*, 650 F.2d 1212, 211 USPQ 323 (CCPA 1981). The examiner should still consider the subject matter added to the claim in making rejections based on prior art since the new matter rejection may be overcome by applicant.

In an instance in which the claims have not been amended, *per se*, but the specification has been amended to add new matter, a rejection of the claims under 35 U.S.C. 112, first paragraph should be made whenever any of the claim limitations are affected by the added material.

When an amendment is filed in reply to an objection or rejection based on 35 U.S.C. 112, first paragraph, a study of the entire application is often necessary to determine whether or not "new matter" is involved. Applicant should therefore specifically point out the support for any amendments made to the disclosure.

APPENDIX B

GeneCard for gene MDM2 GC12P067488

Approved UCL/HGNC/HUGO Human Gene Nomenclature database symbol
MDM2 (Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mous

Aliases and Descriptions
(According to GDB, OMIM, HUGO, LocusLink, SWISS-PROT/TrEMBL, and/or GeneLoc)

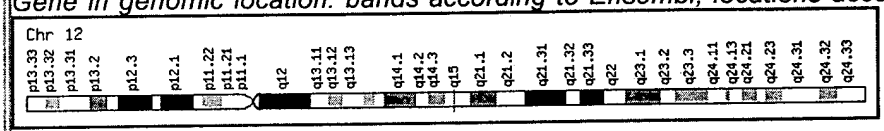
- hdm2 (LL)
- Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse) (GDB, LL)
- mouse double minute 2, human homolog of; p53-binding protein (GDB)
- p53-binding protein MDM2 (LL)
- Ubiquitin-protein ligase E3 Mdm2 (EC 6.3.2.-) (p53-binding protein Mdm2) (Oncop

Previous GC identifers: GC12M068977 GC12P069030 GC12P068918

Chromosomal Location
(According to GeneLoc and/or HUGO, and/or LocusLink (NCBI build 34), Genomic Views According to UCSC and Ensembl)

Chromosome: **12** GeneLoc gene densities
LocusLink cytogenetic band: **12q14.3-q15** *Ensembl cytogenetic band:* **12q15**

Gene in genomic location: bands according to Ensembl, locations according to Gene



GeneLoc location for GC12P067488: (about GC identifiers)

Start:	**67,488,238** bp	from *pter*
End:	**67,520,481** bp	from *pter*
Size:	**32,243** bases	
Orientation:	**plus** strand	

Genomic View:
UCSC Golden Path with GeneCards custom track

Proteins
(According to SWISS-PROT/TrEMBL and/or MIPS, PDB rendering according to OCA)

Swiss-Prot: MDM2_HUMAN

- **Size**: 491 amino acids; 55232 Da
- **Cofactor**: ZINC IS REQUIRED FOR UBIQUITIN LIGASE E3 ACTIVITY.
- **Subunit**: BINDS P53, P73, ARF(P14), RIBOSOMAL PROTEIN L5 AND SPECIFIC WITH RETINOBLASTOMA PROTEIN (RB), E1A-ASSOCIATED PROTEIN P300 AI
- **Subcellular location**: NUCLEAR AND CYTOPLASMIC. EXPRESSED PREDOMIN INTERACTION WITH ARF(P14) RESULTS IN THE LOCALIZATION OF BOTH PR(NUCLEOLAR LOCALIZATION SIGNALS IN BOTH ARF(P14) AND MDM2 MAY BE NUCLEOLAR LOCALIZATION OF BOTH PROTEINS.
- **Alternative products**: Alternative splicing.
- **Post-translational modifications**: PHOSPHORYLATED IN RESPONSE TO IONIZ
- **Miscellaneous**: MDM2 RING FINGER MUTATIONS THAT FAILED TO UBIQUITIN P53 FOR DEGRADATION WHEN EXPRESSED IN CELLS.
- **3D structure:** PDB id 1YCR (**3D**)

MIPS Pedant Viewer: 74112 74106 74107 74108 74109

REFSEQ proteins (6 alternative transcripts): NP_002383.1 NP_006869.1 NP_006870.1 NP_006871.

Protein Domains/ Families
(According to InterPro, ProtoNet, SWISS-PROT/TrEMBL)

InterPro Domains and Families:

IPR001876 Znf_RanGDP
IPR003121 SWIB
IPR001841 Znf_ring

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Genomic organisation of the human *MDM2* oncogene and relationship to its alternatively spliced mRNAs☆

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Abstract

The *MDM2* proto-oncogene, which encodes a protein that binds to the p53 tumour suppressor, has been found amplified and overexpressed in a range of human tumours. Although the human *MDM2* cDNA sequence has been reported, the genomic organisation of the human gene has not been documented. We have previously reported the detection of five alternative internally deleted *MDM2* transcripts in human tumours and suggested these may represent alternatively spliced forms. Here we demonstrate two novel *MDM2* transcripts with internal deletions, using RT-PCR followed by sequencing. To definitively ascribe these variant transcript forms to alternative splicing, and to explore associated mechanisms, we have determined the intron–exon organisation of the human genomic sequence. The human *MDM2* gene spans approximately 33 kb and is divided into 12 exons. Exon sizes range from 50 to ≥ 1161 bp and intron sizes vary from 121 to ~ 7000 bp. The positions of intron–exon boundaries are compared with the deletion junctions of the multiple-sized transcripts and discussed in relation to alternative splicing mechanism.

Keywords: p53; genomic mapping; long range PCR

1. Introduction

The *mdm2* proto-oncogene was initially identified as an amplified gene from a mouse double minute chromosome present in a spontaneously transformed Balb/C 3T3 cell line, 3T3DM (Haines et al., 1994; Sigalas et al., 1996; Steinman et al., 2004). The causal role of this gene in tumorigenesis was originally established by transfection studies using genomic DNA sequences. In these studies, experimental overexpression of *mdm2* resulted in the immortalisation of primary rat embryo fibroblasts and induced a fully transformed phenotype in the cells when cotransfected with an activated *ras* gene (Finlay, 1993). The human homologue of the *MDM2* gene has been found to be amplified in over 30% of human sarcomas (Oliner et al., 1992; Leach et al., 1993), which consequently results in high levels of the *MDM2* gene product. In addition, *MDM2* overexpression can also occur through enhanced transcription and translation (Bueso-Ramos et al., 1993; Landers et al., 1997; Momand et al., 1998).

The human *MDM2* gene has been localised to chromosome 12q13–14. Although the human *MDM2* cDNA sequence has been previously reported (Oliner et al., 1992), little is known about its genomic organisation. The MDM2 protein is composed of 491 amino acids and contains a p53 binding domain (codons 19–102), a putative nuclear localisation signal (codons 181–185), an acidic domain (codons 223–274), a central zinc-finger motif (codons 305–332) and a ring-finger motif towards the C-terminal end of the protein (codons 438–478) (Boddy et al., 1994).

Abbreviations: *MDM2*, mouse double minute 2 gene; RT-PCR, reverse transcription polymerase chain reaction; cDNA, complementary DNA; kb, kilobase; bp, base pair; TE, tris(hydroxymethyl)aminomethane ethylene diamine tetra acetate.

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MDM2 appears to be a pluripotential oncoprotein, exerting its transforming properties through several alternative mechanisms, of which the most extensively studied has been the negative regulation of p53 function. MDM2 blocks p53 transcriptional function by binding to p53 (Momand et al., 1992; Oliner et al., 1993). The binding of MDM2 to p53 also results in the rapid degradation of p53 (Haupt et al., 1997; Kubbutat et al., 1997). In addition, MDM2 has been reported to have p53-independent tumorigenic properties. This includes the ability to interact with and inactivate the pRb tumour suppressor protein (Xiao et al., 1995) and to bind to and activate the E2F1 transcription factor (Martin et al., 1995). Furthermore, two independent transgenic studies have shown *MDM2* to have tumourigenic properties in p53 null mice (Lundgren et al., 1997; Jones et al., 1998).

One of the distinctive properties of *MDM2* is the possession of an extremely complex expression pattern. Its multiple-sized transcripts and proteins have been found in tumour samples and cell lines by a number of groups (Haines et al., 1994; Sigalas et al., 1996; Bartel et al., 2002). In our previous studies, five alternatively sized transcripts of the human *MDM2* were found in human ovarian tumour, bladder tumour and leukaemic cell samples (Sigalas et al., 1996). The expression of the alternatively sized forms was found to be more frequent in tumours of advanced stage and high histological grade, and they also retained their ability to transform NIH3T3 cells. Here, we present data demonstrating two further *MDM2* transcript forms with internal sequence deletions in human tumour tissue. We hypothesised that these transcripts are generated by alternative splicing. To test this hypothesis and to explore the associated mechanisms, we have investigated the genomic structure and organisation of the human *MDM2* gene. This gene is ~ 33 kb in length and comprises at least 12 exons. The sizes of exons vary from 50 to ≥ 1161 bp, and introns range in size from 121 to ~ 7000 bp. The position of intron–exon boundaries is compared with the sequences of the *MDM2* variant transcripts and discussed in relation to alternative splicing mechanisms.

2. Materials and methods

2.1. Nested RT-PCR

Total RNA was extracted from human bladder tumour and normal bladder tissues. Nested RT-PCR was carried out as previously described (Sigalas et al., 1996).

2.2. Genomic DNA extraction

Genomic DNA was prepared from frozen normal human placental tissue by digestion with proteinase K and phenol–chloroform extraction. The DNA was precipitated with a half volume of 7.5M ammonium acetate and one volume of isopropanol, washed in 70% ethanol and resuspended in 1 × TE buffer (10 mM Tris, 0.1 mM EDTA pH 7.5).

2.3. Long-range PCR

Primers (Table 1) were designed from the published *MDM2* cDNA sequence (Oliner et al., 1992). Each primer pair was designed to cross the deletion junctions of multiple-sized transcripts (Sigalas et al., 1996) or according to the predicted exon/intron boundaries by referring to the mouse *mdm2* gene structure (Jones et al., 1996; Montes de Oca luna et al., 1996). A long-range PCR protocol was carried out, with the above human genomic DNA as a template, using an XL PCR Kit (Perkin Elmer, Part No. N808-192). For comparison, PCR was also carried out on normal human placental cDNA. The reaction contained 1 × reaction buffer, 0.8 mM dNTP, 1.1 mM $Mg(OAc)_2$ and 4 units of r*Tth* DNA polymerase, 40 pmol of each primer and 100 ng of genomic DNA or 20 μl cDNA in a total volume of 100 μl. The long-range PCR was performed using a thermal cycler (Perkin-Elmer Model 480) as follows: 94 °C for 2 min; cycles 1–16 at 94 °C for 30 s, 58–62 °C for 10 min; cycles 17–28 at 94 °C for 30 s, 58–62 °C for 10 min and 15 s of increment

Table 1
The sequences of primer pairs for amplification of genomic nucleotides of the human *MDM2* gene

Primer name	Forward primer (5′→3′)	cDNA position (nt)[a]	Reverse primer (5′→3′)	Position at cDNA (nt)[a]	Annealing temperature (°C)
Pri1	cctgtgtggccctgtgtgtc	30–49	tgttccgaagctggaatctgtg	382–361	60
Pri2	gtgcaataccaacatgtctg	314–333	caacagactttaataacttcaaaagc	435–410	60
Pri3	gcttttgaagttattaaagtctgttg	410–435	tacaatatgttgttgcttctcatc	536–513	57
Pri4	ttttatcttggccagtatattatg	474–497	attcctgctgattgactactacc	654–632	57
Pri5	catgatctacaggaacttggtag	614–636	ttgatcactcccaccttcaagg	716–695	60
Pri6	actcaggtacatctgtgagtgag	661–683	tgtctcactaattgctctccttc	815–793	60
Pri7	ttgtacaagagcttcaggaagag	724–746	atggcgtccctgtagattcac	969–949	60
Pri8	tgaaagcctggctctgtgtg	893–912	caaattctacactaaactgatctg	1059–1036	57
Pri9	tcttgatgctggtgtaagtgaac	980–1002	agctaaggaaatttcaggatcttc	1211–1188	57
Pri10	gtgatacagattcatttgaagaag	1168–1191	catctgttgcaatgtgatggaag	1277–1254	60
Pri11	ctattggaaatgcacttcatgc	1213–1235	cggtggctcatgcctgtaatc	2372–2351	57

[a] Sequence of *MDM2* cDNA clones (Oliner et al., 1992).

per cycle, with a final extension of 72 °C for 10 min after the last cycle.

PCR products were separated by electrophoresis on a 1% low melting temperature agarose gel (NuSieve GTG, Flowgen) and visualised by ethidium bromide staining with UV transillumination. DNA bands amplified from genomic DNA were excised and purified with a QIAquick Gel Extraction kit (Qiagen), by following the procedure recommended by the company.

2.4. Cloning PCR products

Purified PCR products were subcloned directly into the pGEM-T Easy vector (Promega) following the protocol recommended by the company. Ligation products were transformed into *Escherichia coli* JM109 and clones containing the desired inserts were identified by PCR screening. Plasmids were prepared by using the Wizard Plus SV Miniprep system (Promega).

2.5. Sequencing

Sequencing was carried out manually by using PCR product directly as a template or automatically by using plasmid PCR product clone as a template. Manual sequencing was performed using the Sequenase Version 2.0 DNA sequencing system (Amersham, Product No.70770). The automated sequencing was carried out in the central core facility at the University of Newcastle upon Tyne Medical Faculty. The sequences were aligned with the published *MDM2* cDNA sequence, using the DNASTAR sequence analysis software package.

3. Results

3.1. The detection of multiple-sized MDM2 transcripts in human tumours

In our previous studies, we have found five alternative-sized *MDM2* transcripts (*MDM2-a*, *-b*, *-c*, *-d* and *-e*) (Sigalas et al., 1996). Our present investigation of the *MDM2* transcriptional pattern in human bladder tumour samples, but not in normal bladder tissue (data not shown), using RT-PCR has revealed two further transcripts, sized 813 and 707 bp, which we have designated *MDM2-a1* and *-g* (Fig. 1). Sequencing shows that these two transcripts have internal sequence deletions: *MDM2-a1* lacks nucleotides from codons 28 to 222 and codons 275 to 300; *MDM2-g* misses nucleotide codons from 28 to $97^2/_3$ and $114^2/_3$ to 300. Fig. 2 shows the structure of these transcripts in relation to the full-length *MDM2* cDNA sequence and previously described variant transcripts (see GenBank accession numbers AF201370 and AF201371 for details of the sequences).

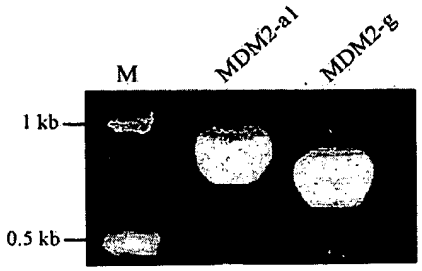

Fig. 1. *MDM2* transcripts were amplified by RT-PCR with nested primers that flank the *MDM2* coding region, as previously described (Sigalas et al., 1996; Matsumoto et al., 1998). M: molecular weight maker.

3.2. MDM2 genomic structure and organisation

We have used long-range PCR amplification, followed by cloning and sequencing to investigate the organisation of the human *MDM2* gene and in particular to define intron–exon boundaries and flanking intronic sequences. Eleven DNA fragments were amplified from genomic DNA with the primer pairs shown in Table 1, which match to the known *MDM2* cDNA sequences. Comparison of the sequences of these PCR products with the published sequences of *MDM2* cDNA clones reveals that the Pri1 primer pair spans introns 1 and 2; while primer pairs Pri2–10 cover one intron per primer pair. However, the region flanked by primer pair Pri11, covering from the 1235th to 2351st nucleotide of the *MDM2* cDNA clone sequence (Oliner et al., 1992), was found not to contain any intronic sequence. Sequence analysis indicates that *MDM2* spans approximately 33 kb of genomic DNA and is separated by 11 introns. Exons range in size from 50 to ≥ 1161 bp. The size of the introns varies from 121 to ~ 7000 bp (Table 2). Exon-intron boundary sequences of the 5′ and 3′ splice sites follow the "GT and AG" rule (Table 3; see GenBank accession numbers AF144014 AF144033 for additional intronic sequence data). Table 4 shows the 3′ ends of the intronic sequences adjacent to the intron–exon boundaries, including branch sites and polypyrimidine tracts. The sequences of branch sites have a good match with the consensus sequence YURA*Y (Y: pyrimidine; R: purine; A*: branch point residue). The distances between the branch points and the 3′ splice sites vary from 18 to 111 bp. The C/T content in the polypyrimidine tracts ranges from 53% to 90%.

Comparison of the structure and organisation of the human *MDM2* gene described here with that published for the mouse gene (Jones et al., 1996) indicates that the number of the exons and introns is the same. The size of the coding exons is similar (Table 2). However, the sizes of the noncoding transcribed regions, including exons 1, 2 and 12 and the introns, differ substantially, with the exception of introns 1 and 3.

3.3. Analysis of alternatively spliced variants of MDM2 mRNA

We have detected seven *MDM2* transcript variants (*MDM2-a*, *-a1*, *-b*, *-c*, *-d*, *-e* and *-g*) previously and

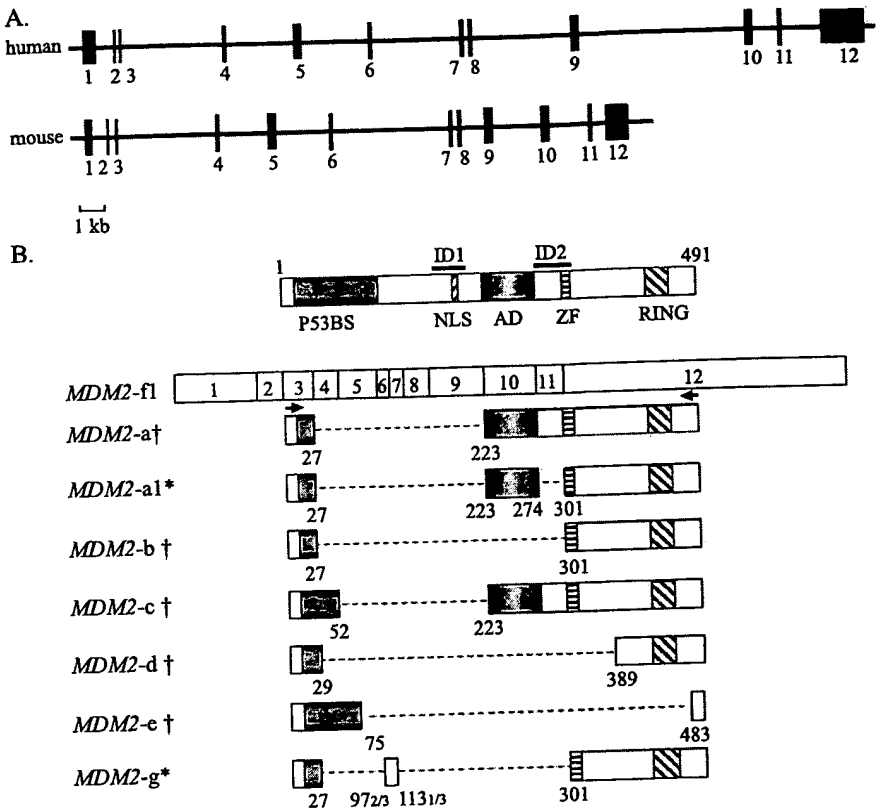

Fig. 2. (A) Genomic organisation of the *MDM2* locus. The top penal presents schematically the human *MDM2* gene structure. For comparison, the mouse *mdm2* gene is illustrated in the bottom panel (Jones et al., 1996). Exons are depicted as vertical bars and numbered underneath. Introns are indicated by horizontal line. (B) The relationship between the various *MDM2* transcripts and the exon–intron boundaries. The second top strip shows the exon organisation of the human *MDM2* gene with its encoded protein above the corresponding exons (top strip). Nucleotide sequence coding for the p53 binding site (p53 BS), nuclear localisation signal (NLS), acidic domain (AD), zinc-finger (ZF), ring-finger (RING) and growth-inhibitory domains 1 and 2 (ID1 and ID2) are depicted with filled boxes. The multiple-sized transcripts that were amplified by RT-PCR with a primer pair (denoted by arrows) are shown with boxes. Dashed lines represent deleted nucleotide sequences. The deletion junctions are numbered by amino acid codons. *detected in this report. †reported by Sigalas et al., 1996.

presently (Sigalas et al., 1996). Some of these variant transcripts have also been demonstrated in astrocytic neoplasms by another group (Matsumoto et al., 1998).

Table 2
Comparison of the human *MDM2* structure with the mouse gene

Exon no.	Exon size (bp)		Intron no.	Intron size (bp)		
	Mouse[a]	Human		Mouse[a]	Human	Difference in size[b] (%)
Exon 1	115	307	Intron 1	~ 500	~ 540	7.4
Exon 2	82	71	Intron 2	160	121	24.4
Exon 3	85	85	Intron 3	~ 4000	~ 4200	5
Exon 4	75	75	Intron 4	~ 2000	~ 3200	37.5
Exon 5	134	134	Intron 5	~ 1750	~ 3500	50
Exon 6	50	50	Intron 6	~ 5100	~ 4000	20
Exon 7	59	68	Intron 7	331	124	62.5
Exon 8	97	97	Intron 8	~ 950	~ 4200	77
Exon 9	164	161	Intron 9	~ 1800	~ 7000	74.3
Exon 10	156	156	Intron 10	~ 1300	~ 900	30.8
Exon 11	78	78	Intron 11	~ 450	~ 2800	83
Exon 12	≥615	≥1161				

[a] Reference: (Jones et al., 1996).
[b] = bp of difference between mouse and human/bp of the larger corresponding intron.

The alignment of the genomic sequence with the sequences of these seven transcripts discloses that the multiple-sized transcripts resulted from the deletion of multiple entire exons or part of exon sequences (Fig. 2). *MDM2-a* skips exons 4 to 9; *MDM2-a1*, exons 4 to 9 and exon 11; *MDM2-b*, exons 4 to 11; *MDM2-c*, exons 5 to 9; *MDM2-d*, exons 5 to 11, most of exon 4 and the 5′ end of exon 12; *MDM2-e*, exons 6 to 11, the 3′ end of exon 5 and the 5′ end of exon 12. *MDM2-g* splices out exons 4 to 5, and exons 7 to 11 (Fig. 2) Among the skipped exons, exons 4 to 9 and exon 11 were most frequently missed. These commonly excluded exons (with the exception of exon 7) have either less than 60% of C/T content at their upstream polypyrimidine tracts and/or more than 40 bp between their upstream branch points and 3′ sites. Whereas, exons 3, 10 and 12, which are most frequently included in the transcripts, have higher than 60% C/T content within their upstream polypyrimidine tracts and less than 40 bp between their upstream branch points and 3′ splice sites. Since exon 6 contains interrupted codons at both ends, the *MDM2-g* variant, which splices out several exons up- and downstream of

Table 3
Sequence of the human *MDM2* exon/intron boundaries

Exon no.	Sequence of boundary	Exon no.
1	GGAGCAGgtgctggc--intron 1--tttcccagCTGTGTT	2
2	GATCCAGgtaagcac--intron 2--ccttgtagGCAAATG 1	3
3	GACCCTGgttagtat--intron 3--tcttatagGTTAGAC 27 28	4
4	GAAAGAGgtaagctg--intron 4--tatttcagGTTCTTT 52 53	5
5	AGCACAGgtaattct--intron 5--tctacaagGAAAATA 96 98	6
6	CAGCAGGgtaagtta--intron 6--tctctcagAATCATC 113 115	7
7	TCAAAAGgtaatcta--intron 7--atgcttagGACCTTG 136 137	8
8	GAGACAGgtatatat--intron 8--atatccagAAGAAAA 167 169	9
9	GAATCCGgtaatgtt--intron 9--tgttttagGATCTTG 222 223	10
10	TGATGAGgtatatat--intron 10-tttattagGTATATc 274 275	11
11	CTTAGCTgtaagtat--intron 11-cattgaagGACTATT 300 301	12

Exon sequences are shown as uppercase letters. Intron sequences are denoted by lowercase letters. Underlined nucleotides encode amino acids (numbered underneath).

exon 6, has a shifted reading frame after codon 28. The *MDM2-e* variant, which also involves a deletion junction with an interrupted codon sequence, has a shift in the reading frame after codon 484.

4. Discussion

In our previous studies and the results presented here, we detected multiple-sized *MDM2* transcripts in human

Table 4
Branch sites and polypyrimidine tracts of the human *MDM2* gene

Intron no.	Sequences of branch site and polypyrimidine tract (5′ → 3′)	Distance from branch point to 3′ site (nt)	T and C %[a]
1	ctgacttgtctccagctggggctatttaaaccatgcattttcccag/exon 2	>40	59
2	atgatttccagttttcatcgtgtctttttttttccttgtag/exon 3	<40	78
3	ttgatggatatgtttgctgcagggcctatagttctgggataatttttggaagtataatagcagttctttttctctttatag/exon 4	>40	53
4	ctaacatttagttatctttaatgctcagaatcatatttgtatttcag/exon 5	>40	60.5
5	ttaatacaaatttttattctaaaatgtacatctcttgttatttttttttttttctgtctacaag/exon 6	>40	68
6	ctgatccttttttcttttctctcag/exon 7	<40	90
7	ctaatgtaatattatttgcaaattggaaaggttatttcaacaagttagcttactggttatgttaagtttgttgtattttattttttttcctaaatgcttag/exon 8	>40	56.3
8	ttaacttttagaactattttattgaaactaagtttctgtacaaataggtactcaaaacagctcaattttctaaatcacagtacagcaatttattttttttccttacatatccag/exon 9	>40	57
9	gtgatgtttatcaaattatatatttttttcttgttttag/exon 10	<40	69
10	ctaatgaatgtgttttattag/exon 11	<40	53
11	ctgactgtgtgtcttatttcattgaag/exon 12	<40	61

Branch site sequences are indicated with underlined letters. /: boundary between intron and exon.
[a] The percentage of T and C content in polypyrimidine tracts.

tumour tissues, but not in normal tissues. Detection of alternative spliced forms of *MDM2* mRNAs varied, and appeared to be relatively abundant in most samples. These variants encoded protein products in vitro and were found to transform NIH3T3 cells and to be associated with high-grade and late-stage human cancer (Sigalas et al., 1996 and unpublished data). Our data were supported by the observations of other groups, who reported that alternatively spliced *mdm2* transcripts promoted tumour formation in mouse model (Fridman et al., 2003; Steinman et al., 2004). These collective observations suggest that alternatively spliced forms of *MDM2*, encoding alternative proteins with differing functional capabilities, may play an important role in tumour development. The human *MDM2* genomic map presented here enable us to relate the genomic structure and organisation of *MDM2* to the appearance of variant transcript forms and provide a basis for considering alternative splicing mechanisms.

Pre-mRNA splicing involves precise excision of intron sequences and the ligation of exon sequences. In cases of alternative splicing, the excision may occur at cryptic splice sites; exons may be skipped and introns may be retained. The organisation of the exon–intron boundaries of the human *MDM2* gene indicates that the *MDM2-a*, *-a1*, *-b*, *-c* and *-g* variant transcript forms (Fig. 2) result from multiple entire exon skipping, because the internal deletion junctions correspond exactly to the location of exon–intron boundaries. However, the deletion junctions of *MDM2-d* and *-e* forms do not correspond to the boundaries between exons and introns, and there are no consensus splicing sequences surrounding them to indicate the possible use of cryptic splice sites. This suggests that they may have resulted from an unusual and possibly aberrant splicing mechanism.

The regulation of alternative splicing involves both *cis* elements and *trans*-acting factors. The *cis* elements include the 5′ and 3′ splice sites, a branch site and a polypyrimidine tract between the branch point and the 3′ splice site. It has been demonstrated that a short distance between the branch point and the 3′ splice site and high C/T content at polypyrimidine tracts give rise to high efficiency of splicing in mammals (Helfman et al., 1988; Libri et al., 1989). Our data show that all the 5′ and 3′ splice sites of the human *MDM2* gene obey the "GT" and "AG" rule. The branch sites also have a good match with the consensus sequence. However, the distances between branch points and 3′ sites, and the percentage C/T content vary between introns. The exons most frequently retained in the splice variants have shorter distances between their upstream branch points and 3′ splice sites and/or a higher percentage C/T content in their upstream polypyrimidine tracts, compared with those exons commonly excluded (Table 2). It suggests that the short distance between the branch point to the 3′ site and the polypyrimidine tract with high C/T content confer high splicing efficiency to their adjacent splicing sites, which is consistent with the observation reported by Smith et al. (1989), Goux-Pelletan et al. (1990), Mueller et al. (1997). However, exon 7 was spliced out with a high frequency although there is only a short distance between its branch point and 3′ splice site and there is also a high C/T content in its upstream polypyrimidine tract. The explanation may be that it is spliced out along with upstream exons. The order of intron removal is governed by preferential binding of splice factors rather than in a sequential numerical order (Lewin, 1994). It may be possible that exon 7 processes splicing before exon 6 does, and the 5′ end of exon 7 may in some circumstances be ligated preferentially to the 3′ end of exon 6. If exon 6 has low splice strength, then in the process of ligation with its upstream exon, it may be outcompeted and skipped out together with exon 7.

Currently, we are not clear why the alternative spliced transcripts appear preferentially in tumour samples, especially in advanced stage and high grade, but not in normal tissues (Sigalas et al., 1996; Bartel et al., 2002). Although variant *MDM2* spliced transcripts have been reported in normal tissues in one study (Bartel et al., 2004), we failed to detect these isoforms in noncancerous tissues. It has been proposed that a mRNA surveillance system exists in cells, which protects them from errors of transcription, mRNA processing, or mRNA transport (Pulak and Anderson, 1993). Mistakes are not uncommon in splicing of RNA from complex genes. Exons can occasionally be skipped (Nigro et al., 1991). In the normal situation, the surveillance system would probably degrade most mRNA with splicing errors as they are transported to the cytoplasm. We speculate that in cancer cells, this system may not function correctly and, consequently, the splice variants may escape degradation. It is also possible that there are mutations in the intron region that cause alternative splicing and the presence of the variants contribute to the cancer. It would be of interest to test this hypothesis by investigating intron nucleotide sequences in tumour samples that show expression of alternatively spliced forms. However, other models can be envisaged; for instance, we cannot rule out the possibility that *trans*-acting factors are involved in the alternative RNA processing by blocking some splice sites and/or enhancing other splice sites.

In conclusion, we have detected two novel *MDM2* alternatively spliced transcripts and have also defined the structure and organisation of the human *MDM2* gene. In addition we have related this information to potential mechanisms by which alternatively sized *MDM2* transcripts are generated. As the alternatively spliced *MDM2* mRNAs have been shown to possess oncogenic potential and to correlate with advanced malignancies to tumour progress (Haines et al., 1994; Sigalas et al., 1996; Steinman et al., in press), our data may assist in clinical diagnosis of sarcomas displaying *MDM2* amplification and alternative splicing of *MDM2* transcripts.

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